

What is claimed is:

1. A multimer assembly of DNA sequences comprising:  
at least one amplification cassette, wherein said at least one amplification cassette  
5 comprises at least one monomer sequence whose polymerization is desired,  
further wherein said at least one amplification cassette comprises a 5' restriction  
pair member at its 5' terminus and a 3' restriction pair member at its 3' terminus;  
and at least one of the following:  
at least one 3'-terminal cassette, wherein said 3'-terminal cassette  
10 comprises at least one 3' specific sequence and a 5' restriction pair  
member site that can be fused to a 3' restriction pair member site of at  
least one of said at least one amplification cassette; or  
at least one 5'-terminal cassette, wherein said 5'-terminal cassette  
comprises at least one 5' specific sequence and a 3' restriction pair  
15 member site that can be fused to a 5' restriction pair member site of at  
least one of said at least one amplification cassette.
2. The multimer assembly of claim 1, wherein said at least one amplification  
cassette is at least two amplification cassettes.
- 20 3. The multimer assembly of claim 2, wherein said at least two amplification  
cassettes are fused at restriction pair member partners.
4. The multimer assembly of claim 1, wherein said multimer assembly comprises  
25 said at least one 5'-terminal cassette.
5. The multimer assembly of claim 1, wherein said multimer assembly comprises at  
least one 3'-terminal cassette.
- 30 6. The multimer assembly of claim 5, wherein said multimer assembly further  
comprises at least one 5'-terminal cassette.

7. The multimer assembly of claim 1, wherein said 5' restriction pair member site and said 3' restriction pair member site comprise:
- 5           ligation-compatible non-regenerable overhang restriction sites;  
            ligation-compatible non-regenerable blunt end restriction sites; or  
            incompatible overhang restriction sites that are converted to ligation-compatible non-regenerable blunt end restriction sites through the use of polymerases or nucleases.
- 10   8. The multimer assembly of claim 7, wherein said 5' restriction pair member site and said 3' restriction pair member site comprise ligation-compatible non-regenerable overhang restriction sites.
- 15   9. The multimer assembly of claim 7, wherein said 5' restriction pair member site and said 3' restriction pair member site comprise ligation-compatible non-regenerable blunt end restriction sites.
- 20   10. The multimer assembly of claim 7, wherein said 5' restriction pair member site and said 3' restriction pair member site comprise incompatible overhang restriction sites that are converted to ligation-compatible non-regenerable blunt end restriction sites through the use of polymerases or nucleases.
- 25   11. The multimer assembly of claim 4, wherein said 5'-terminal cassette further comprises at least a portion of said monomer sequence.
- 30   12. The multimer assembly of claim 5, wherein said 3'-terminal cassette further comprises at least a portion of said monomer sequence.
13. The multimer assembly of claim 6, wherein said 3'-terminal cassette and said 5'-terminal cassette each comprise at least a portion of said monomer sequence.

14. The multimer assembly of claim 1, wherein said multimer assembly further comprises at least one linker.
15. The multimer assembly of claim 14, wherein said at least one linker comprises at least one restriction pair member.
16. The multimer assembly of claim 1, wherein said monomer sequence encodes a peptide or protein of interest.
17. The multimer assembly of claim 16, wherein said peptide or protein of interest comprises at least a portion of a diagnostic protein.
18. The multimer assembly of claim 17, wherein said diagnostic protein is a cytokine, a hormone, a receptor, a receptor ligand, an enzyme, an inhibitor, a transcription factor, a translation factor, a DNA replication factor, an activator, a chaperone, or an antibody.
19. The multimer assembly of claim 16, wherein said peptide or protein of interest comprises at least a portion of a therapeutic protein.
20. The multimer assembly of claim 19, wherein said therapeutic protein is a cytokine, a growth factor, a hormone, a receptor, a receptor ligand, an enzyme, an inhibitor, a transcription factor, a translation factor, a DNA replication factor, an activator, a chaperonin, or an antibody.

21. The multimer assembly of claim 20, wherein said therapeutic protein is Interferon alpha., Interferon-beta., Interferon-gamma., Interleukin-1, Interleukin-2, Interleukin-3, Interleukin-4, Interleukin-5, Interleukin-6, Interleukin-7, Interleukin-8, Interleukin-9, Interleukin-10, Interleukin-11, Interleukin-12, Interleukin-13, Interleukin-14, Interleukin-15, Interleukin-16, Erythropoietin, Colony-Stimulating Factor-1, Granulocyte Colony-stimulating Factor, Granulocyte-Macrophage Colony-Stimulating Factor, Leukemia Inhibitory Factor, Tumor Necrosis Factor, Lymphotoxin, Platelet-Derived Growth Factor, Fibroblast Growth Factors, Vascular Endothelial Cell Growth Factor, Epidermal Growth Factor, Transforming Growth Factor-beta., Transforming Growth Factor-alpha., Thrombopoietin, Stem Cell Factor, Oncostatin M, Amphiregulin, Mullerian-Inhibiting Substance, B-Cell Growth Factor, Macrophage Migration Inhibiting Factor, Endostatin, or Angiostatin.
22. The multimer assembly of claim 1, wherein said 3'-restriction pair member encodes a stop codon that is destroyed upon ligation to said 5'-restriction pair member.
23. An amplification cassette comprising a 5' segment of a monomer sequence and a 3' segment of a monomer sequence that together comprise the sequence of a complete monomer, wherein said 5' segment is positioned 3' of said 3' segment, further wherein 5'terminus of said 3' segment is a 5' restriction pair member and the 3' terminus of said 5' segment is a 3' restriction pair member.
24. The multimer assembly of claim 1, wherein said multimer assembly comprises an amplification cassette that comprises a 5' segment of a monomer sequence and a 3' segment of a monomer sequence that together comprise the sequence of a complete monomer, wherein said 5' segment is positioned 3' of said 3' segment, further wherein 5'terminus of said 3' segment is a 5' restriction pair member and the 3' terminus of said 5' segment is a 3' restriction pair member.

25. The multimer assembly of claim 24, wherein said multimer assembly comprises:

at least one amplification cassette, wherein said at least one amplification  
cassette comprises at least one monomer sequence whose polymerization is  
desired, further wherein said at least one amplification cassette comprises a 5'  
restriction pair member at its 5' terminus and a 3' restriction pair member at  
its 3' terminus;

a 3'-terminal cassette, wherein said 3'-terminal cassette comprises said 3'  
segment; and

a 5'-terminal cassette, wherein said 5'-terminal cassette comprises said 5'  
segment.

26. The multimer assembly of claim 24, wherein said amplification cassette  
comprises a linker that is positioned between said 5' segment and said 3' segment  
of said monomer sequence.

27. The multimer assembly of claim 1, wherein said multimer assembly comprises a  
first cassette and a second cassette,

wherein when said first cassette comprises a 5'-terminal cassette, said  
second cassette comprises an amplification cassette or a multimer cassette  
constructed from a 3'-terminal cassette and an amplification cassette;  
and when said first cassette comprises a 3'-terminal cassette, said second  
cassette comprises an amplification cassette or a multimer cassette  
constructed from a 5'-terminal cassette and an amplification cassette.

28. A method of making a multimer cassette from a multimer assembly of claim 27, comprising:
- a) digesting said first cassette at said 5'-restriction pair member or said 3'-restriction pair member and isolating a first fragment containing the insert sequence from said first cassette;
  - b) digesting said second cassette at said 5' restriction pair member site and said 3' restriction pair member site and isolating a second fragment containing the insert sequence from said second cassette;
  - c) ligating said first fragment with said second fragment to generate multimer cassette candidates; and
  - d) testing said multimer cassette candidates for correct ligation orientation, wherein a multimer cassette candidate with correct ligation orientation comprises a multimer cassette.
29. A multimer cassette made by the method of claim 28.
30. The method of claim 28 wherein said first cassette is a 3'-terminal cassette and said second cassette is an amplification cassette.
31. A multimer cassette made by the method of claim 30.
32. The method of claim 28 wherein said first cassette is a 5'-terminal cassette and said second cassette is an amplification cassette.
33. A multimer cassette made by the method of claim 32.
34. The method of claim 28 wherein said first cassette is a 3'-terminal cassette and said second cassette is a multimer cassette constructed from a 5'-terminal cassette and an amplification cassette.
35. A multimer cassette made by the method of claim 34.

36. The method of claim 28 wherein said first cassette is a 5'-terminal cassette and said second cassette is a multimer cassette constructed from a 3'-terminal cassette and an amplification cassette.

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37. A multimer cassette made by the method of claim 36.

38. The multimer assembly of claim 1, wherein at least one of said cassettes comprises one or more flanking restriction sites.

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39. The multimer assembly of claim 4, wherein at least one of said cassettes comprises one or more flanking restriction sites.

40. The multimer assembly of claim 5, wherein at least one of said cassettes comprises one or more flanking restriction sites.

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41. The multimer assembly of claim 6, wherein at least one of said cassettes comprises one or more flanking restriction sites.

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42. The multimer assembly of claim 6, wherein said 5'-terminal cassette and said 3'-terminal cassette each contain the same insertion restriction site.

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43. A method of making a multimer cassette from two cassettes from a multimer assembly of claim 27, wherein each of said two cassettes comprises one or more flanking restriction sites, comprising:

- 5           a) providing a first cassette with a first flanking restriction site at one end, either 5' or 3', of its insert sequence;
- b) providing a second cassette with a second flanking restriction site that is, or is made, ligation compatible with said first flanking restriction  
10           site and is on the same side, either 5' or 3', of its insert sequence as the first flanking restriction site is relative to said first cassette's insert sequence;
- c) digesting said first cassette at its restriction pair member and said first  
15           flanking site and isolating the first fragment containing the insert sequence;
- d) digesting said second cassette at its restriction pair member partner to said first cassette's restriction pair member and at said second flanking  
20           site and isolating the second fragment containing the insert sequence; and
- e) ligating said first fragment with said second fragment to generate a multimer cassette.

25           44. A multimer cassette made by the method of claim 43.

          45. The method of claim 43 wherein said first cassette is a 3'-terminal cassette and said second cassette is an amplification cassette.



46. The method of claim 43, wherein said first cassette is a 5'-terminal cassette and said second cassette is an amplification cassette.
47. The method of claim 43 wherein said first cassette is a 3'-terminal cassette and said second cassette is a multimer cassette constructed from a 5'-terminal cassette and an amplification cassette.
48. The method of claim 43 wherein said first cassette is a 5'-terminal cassette and said second cassette is a multimer cassette constructed from a 3'-terminal cassette and an amplification cassette.

49. A method of making an insertion cassette from the multimer assembly of claim 42 comprising:

- 5 a) providing said 5'-terminal cassette having a first flanking restriction site, distinct from said insertion restriction site, that is outside of the sequence including the insert sequence and insertion restriction site of said 5'-terminal cassette;
- 10 b) providing a 3'-terminal cassette having a second flanking restriction site, distinct from said insertion restriction site, that is outside of the sequence including the insert sequence and insertion restriction site of said 3'-terminal cassette and is , or is made, ligation compatible with said first flanking site and is on the same side, either 5' or 3', of its insert sequence as the first flanking restriction site is relative to said 5'-terminal cassette's insert sequence;
- 15 c) digesting said 5'-terminal cassette at its insertion restriction site and said first flanking site and isolating the first fragment containing the insert sequence;
- d) digesting said 3'-terminal cassette at its insertion restriction site and said second flanking site and isolating the second fragment containing the insert sequence; and
- 20 e) ligating said first fragment with said second fragment to generate an insertion cassette.

50. An insertion cassette made by the method of claim 49.

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51. A method of making a multimer cassette from a multimer assembly that comprises an amplification cassette and the insertion cassette of claim 50, comprising:

- 5 a) digesting said insertion cassette at both its restriction pair member sites and isolating a first fragment containing an insertion cassette insert sequence;
- b) digesting said amplification cassette at both its said restriction pair member sites and isolating a second fragment containing an amplification cassette insert sequence; and
- 10 c) ligating said first fragment with said second fragment to generate multimer cassette candidates;
- d) testing said multimer cassette candidates for correct ligation orientation, wherein said a multimer cassette candidate with correct
- 15 ligation orientation comprises a multimer cassette.

52. A multimer cassette made by the method of claim 51.

53. A method of making a multimer cassette from a multimer assembly that comprises an amplification cassette and the insertion cassette of claim 50, comprising:

- 5 a) providing said amplification cassette comprising a flanking restriction site that is, or is made, ligation compatible to said insertion restriction site of said insertion cassette;
- b) digesting said amplification cassette at said flanking restriction site and its restriction pair member on the opposite side, either 5' or 3', of the  
10 insert sequence and isolating the first fragment containing the insert sequence;
- c) digesting said insertion cassette at said insertion restriction site and the restriction pair member partner to said digested amplification cassette's restriction pair member and isolating the second fragment containing the  
15 insert sequence;
- d) ligating said first fragment with said second fragment to generate a multimer cassette precursor; and
- e) digesting said multimer cassette precursor at both restriction pair members, isolating the fragment containing the insert sequence, and  
20 ligating it with itself to generate a multimer cassette.

54. A multimer cassette made by the method of claim 53.

55. A multimer assembly according to claim 1, wherein said monomer sequence is  
25 the hGH coding sequence, SEQ ID NO:1.

56. The multimer assembly of claim 25, wherein said monomer sequence is the hGH coding sequence, SEQ ID NO:1.

57. The multimer assembly of claim 56, wherein a restriction pair is utilized at the coding sequence of amino acids 187 and 188, glycine and serine, of monomeric hGH.
- 5 58. The multimer assembly of claim 57, wherein insert sequences lack linkers.
59. The multimer assembly of claim 58, wherein said 5'-terminal cassette is listed in SEQ ID NO: 15.
- 10 60. The multimer assembly of claim 58, wherein said 5'-terminal cassette is listed in SEQ ID NO: 17.
61. The multimer assembly of claim 58, wherein the general formula for said amplification cassette is listed in SEQ ID NO: 28.
- 15 62. The multimer assembly of claim 58, wherein said 3'-terminal cassette is listed in SEQ ID NO: 22.
63. The multimer assembly of claim 58, wherein a 3'-terminal cassette is listed in SEQ ID NO: 24.
- 20 64. The multimer assembly of claim 58, wherein the insertion cassette is listed in SEQ ID NO: 30.
- 25 65. The multimer assembly of claim 58, wherein the general formula for the multimer expression cassettes is listed in SEQ ID NO: 31.
66. A multimer expression cassette made from the multimer assembly of claim 58.
- 30 67. A polymeric protein expressed from the multimer expression cassette of claim 66 as described by SEQ ID NO:32.

68. The multimer assembly of claim 57, wherein at least one insert sequence comprises at least one linker.
- 5 69. The multimer assembly according to claim 68, wherein said linker is the single amino acid glycine.
70. The multimer assembly of claim 69, wherein said 5'-terminal cassette is listed in SEQ ID NO: 15.
- 10 71. The multimer assembly of claim 69, wherein said 5'-terminal cassette is listed in SEQ ID NO: 17.
72. The multimer assembly of claim 69, wherein the general formula for the  
15 amplification cassettes is listed in SEQ ID NO:36.
73. The multimer assembly of claim 69, wherein a 3'-terminal cassette is listed in SEQ ID NO: 22.
- 20 74. The multimer assembly of claim 69, wherein said 3'-terminal cassette is listed in SEQ ID NO: 24.
75. The multimer assembly of claim 69, wherein the insertion cassette is listed in  
25 SEQ ID NO: 30.
76. The multimer assembly of claim 69, wherein the general formula for the  
amplification cassettes is listed in SEQ ID NO:38.
77. A multimer expression cassette made from the multimer assembly of claim 69.  
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78. A polymeric protein expressed from the multimer expression cassette of claim 77 as described by SEQ ID NO:39.
- 5 79. The multimer assembly of claim 22, wherein at least one said insert sequence comprises a linker coding for the peptide sequence A-Ser-Trp-B, where A and B are arbitrary peptide sequences, the 3'-restriction pair member is RcaI, T<sup>^</sup>CATGA, and the 5'-restriction pair member is NcoI, C<sup>^</sup>CATGG.
- 10 80. A multimer assembly according to claim 79, wherein said monomer sequence encodes hGH or a portion thereof.
81. The multimer assembly of claim 80, wherein said linker codes for the peptide Ser-Trp-Gly-Gly-Gly-Gly-Ser.
- 15 82. The multimer assembly of claim 80, wherein a 5'-terminal cassette is listed in SEQ ID NO: 41.
83. The multimer assembly of claim 80, wherein the general formula for the amplification cassettes is listed in SEQ ID NO:48.
- 20 84. The multimer assembly of claim 80, wherein a 3'-terminal cassette is listed in SEQ ID NO: 46.
85. The multimer assembly of claim 80, wherein the general formula for multimer expression cassettes is listed in SEQ ID NO: 52.
- 25 86. The multimer assembly of claim 80, wherein the general formula for multimer expression cassettes is listed in SEQ ID NO: 54.
- 30 87. A multimer expression cassette made from the multimer assembly of claim 80.

88. A polymeric protein expressed from the multimer expression cassette of claim 86 as described by SEQ ID NO:53.
89. A polymeric protein expressed from the multimer expression cassette of claim 86 as described by SEQ ID NO:55.
90. A multimer assembly according to claim 1 comprising at least one linker sequence adjacent to at least one monomer sequence of at least one amplification cassette.
91. The multimer assembly according to claim 5, further comprising at least one linker sequence adjacent to at least one monomer sequence of at least one amplification cassette.
92. The multimer assembly according to claim 91, wherein said linker comprises at least one restriction site compatible with a restriction site of said 3'-terminal cassette.
93. The multimer assembly according to claim 4, further comprising at least one linker sequence adjacent to at least one monomer sequence of at least one amplification cassette.
94. The multimer assembly according to claim 93, wherein said linker comprises at least one restriction site compatible with a restriction site of said 5'-terminal cassette.
95. The multimer assembly according to claim 94, wherein said linker codes for the peptide sequence (GZGS)<sub>x</sub>, where Z is an arbitrary sequence of arbitrary length and x indicates the degree of polymerization of the peptide monomer sequence.
96. The multimer assembly according to claim 95, wherein Z is GG.



97. The multimer assembly of claim 96, wherein a 5'-terminal cassette is listed in SEQ ID NO: 56
- 5 98. The multimer assembly of claim 96, wherein an amplification cassette is listed in SEQ ID NO: 57
99. The multimer assembly of claim 96, wherein an amplification cassette is listed in SEQ ID NO: 58
- 10 100. The multimer assembly of claim 96, wherein a multimer cassette is listed in SEQ ID NO: 60.
101. The multimer assembly according to claim 15, wherein said monomer sequence encodes hGH or a portion thereof.
- 15 102. The multimer assembly according to claim 101, wherein said linker codes for the peptide (G<sub>4</sub>S)<sub>3</sub>.
- 20 103. The multimer assembly according to claim 102, wherein a 5'-terminal cassette is listed in SEQ ID NO: 71
104. The multimer assembly according to claim 102, wherein the general formula for amplification cassettes is listed in SEQ ID NO: 73,
- 25 105. The multimer assembly according to claim 102, wherein a 3'-terminal cassette is listed in SEQ ID NO: 67.
106. The multimer assembly according to claim 102, wherein a general formula for multimer expression cassettes is listed in SEQ ID NO: 75.
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107. A multimer expression cassette made from the multimer assembly of claim 106.
108. A polymeric protein expressed from the multimer expression cassette of claim 107 as described by SEQ ID NO:76.
- 5 109. The multimer assembly according to claim 10, wherein said monomer sequence encodes hGH or a portion thereof.
- 10 110. The multimer assembly of claim 109, wherein a 5'-terminal cassette is listed in SEQ ID NO: 77.
111. The multimer assembly of claim 110, wherein a general formula for said amplification cassettes is listed in SEQ ID NO:83.
- 15 112. The multimer assembly of claim 110, wherein a 3'-terminal cassette is listed in SEQ ID NO: 81.
113. The multimer assembly of claim 110, wherein a general formula for the multimer expression cassettes is listed in SEQ ID NO: 85.
- 20 114. A multimer expression cassette made from the multimer assembly of claim 113.
115. A polymeric protein expressed from the multimer expression cassette of claim 114 as described by SEQ ID NO:86.
- 25 116. A multimer cassette made by the method of claim 45.
117. A multimer cassette made by the method of claim 46.
- 30 118. A multimer cassette made by the method of claim 47.

119. A multimer cassette made by the method of claim 48.
120. A vector comprising a multimer cassette made from a multimer assembly of any of claims 1-27, 38-42, 55-65, 68-76, 79-86, 90-106, or 109-113.
- 5 121. The vector of claim 120, wherein said vector is an expression vector, wherein said expression vector is designed for *in vitro* or *in vivo* expression.
122. A cell containing a vector according to claim 121.
- 10 123. A polymeric protein expressed from a vector of claim 122.
124. A method of making an amplification cassette, comprising:
- 15 a) providing at least two amplification cassettes of claim 23; and
- b) joining said at least two amplification cassettes by ligating said 3' restriction member of at least one of said at least two amplification cassettes to said 5' restriction member of at least one other of said at least two amplification cassettes to generate a multimer cassette.
- 20 125. An amplification cassette comprising at least one linker, wherein said at least one linker comprises at least one restriction pair member member.

126. A method of making an amplification cassette, comprising:
- a) providing at least two amplification cassettes of claim 125; wherein each of said at least two amplification cassettes comprises: a first restriction pair partner on one end of said monomer sequence; and a linker at the other end of said monomer sequence that comprises a second restriction pair partner; and
  - b) joining said at least two amplification cassettes by ligating said first restriction pair partner of at least one of said at least two amplification cassettes to said second restriction pair partner of at least one other of said at least two amplification cassettes to generate a multimer cassette.
127. An amplification cassette comprising 5' and 3' restriction pair member sites that are incompatible overhang restriction sites that are converted to ligation-compatible nonregenerable blunt end restriction sites through the use of polymerases or nucleases.
128. A method of making an amplification cassette, comprising:
- a) providing at least two amplification cassettes of claim 127; and
  - b) joining said at least two amplification cassettes by ligating said 3' restriction member of at least one of said at least two amplification cassettes to said 5' restriction member of at least one other of said at least two amplification cassettes to generate a multimer cassette.
129. A vector comprising a multimer assembly cassette of any of claims 23, 29, 31, 33, 35, 37, 44, 50, 52, 54, 66, 77, 87, 107, 116-119, 125, or 127.
130. The vector of claim 129, wherein said vector is an expression vector, wherein said expression vector is designed for *in vitro* or *in vivo* expression.
131. A cell containing a vector according to claim 130.
132. A polymeric protein expressed from the vector of claim 131.